REMARKS

Reconsideration of the instant application in view of the present amendment and the following comments is respectfully requested. Claims 1-21, 43-59 and 64-96 are pending in the application and claims 1, 19-21, 43, 64, 80 and 81 have been amended. Support for the amendments may be found in the specification and drawings, for example, at page 64, line 26 through page 65, line 6; at page 17, lines 1-16 and in Figure 4. Drawings Figures 1-13 have been amended to reflect identifying indicia (title of the invention, inventors' names, application number and docket number); Figures 5-13 have been amended merely by presenting in type the labeling that was previously present in handwritten form. Eleven sheets of drawings are presented herewith for approval. No new matter has been added to the application.

DRAWINGS

The Action asserts that formal drawings will be required when the application is allowed, and that correction is required for the labeling of Figures 7-13. The Action also asserts that corrected drawings submitted by applicants on July 9, 2002, could not be located. A complete set of clearly labeled replacement drawings is submitted herewith.

REJECTIONS UNDER 35 U.S.C. §112, SECOND PARAGRAPH

Claims 1-21, 43-59 and 64-96 stand rejected under 35 U.S.C. §112, second paragraph, for alleged indefiniteness. The PTO is unclear regarding what conditions or steps are involved that would permit identification of mitochondrial calcium uniporter activity and mitochondrial uncoupler or mitochondrial respiratory inhibitor activity. More specifically, the PTO asserts that it is unclear whether the term "conditions" encompasses concentrations of certain assay components, or whether instead "conditions" refers to active steps of measuring the recited mitochondrial activities. The PTO also alleges that claims 1-21, 43-59 and 64-96 are confusing where it is assertedly unclear whether or not measurement is made of uniporter activity and mitochondrial uncoupler or repiratory inhibitor activity.

Applicants respectfully traverse these grounds for rejection and submit that the meanings of the instant claims are clear and that the specification fully complies with the

requirements of 35 U.S.C. §112(2). The present invention is directed in pertinent part to the recited methods of identifying an agent, comprising detecting at a plurality of time points the signal generated by the calcium indicator molecule under conditions that permit identification of (i) mitochondrial calcium uniporter activity *and* (ii) mitochondrial uncoupler *or* mitochondrial respiratory inhibitor activity. More specifically, conditions that permit identification of mitochondrial calcium uniporter activity are indicated by a decrease over time in the signal that is proportional to the level of calcium in the cytosol, and conditions that permit identification of mitochondrial uncoupler or respiratory inhibitor activity are indicated by an increase over time in the signal that is proportional to the level of calcium in the cytosol, without repeating the recited step of contacting a sample with a source of calcium cations.

As described in the specification, for example, at page 17, lines 1-16; at page 61, lines 13-22; and at page 64, line 26 through page 66, line 2, the invention provides an assay that is capable of specifically determining mitochondrial calcium uniporter activity, which assay also permits identification of, and thus distinction between, (i) mitochondrial calcium uniporter activity and (ii) mitochondrial uncoupler or mitochondrial respiratory inhibitor activity. Therefore, when the assay is performed according to the method recited by the claims and described in the specification, applicants submit that indeed *conditions* are present which permit identification of (i) mitochondrial calcium uniporter activity *and* (ii) mitochondrial uncoupler *or* mitochondrial respiratory inhibitor activity. In other words, the assay is designed to identify *both* of these activities, and to distinguish one from the other by the profile of the signal that is detected over the recited plurality of time points. However, no further active steps are required to identify either activity. Instead, whether a uniporter, or an uncoupler or respiratory inhibitor activity is present will depend on the nature of the signal that is detected, *i.e.*, whether the signal decreases over time or whether the signal increases over time without the step of contacting having been repeated. Because this signal is proportional to the level of calcium in the cytosol, other assay components (*e.g.*, the sample, the absence or presence of the candidate agent) will determine whether a uniporter, an uncoupler or a respiratory inhibitor activity is detected. The invention method therefore itself provides *conditions that permit identification* of (i) mitochondrial calcium uniporter activity and (ii) mitochondrial uncoupler or mitochondrial

respiratory inhibitor activity, but which, if any, such activity will in fact be detected will depend on the sample and/or the candidate agent.

Applicants respectfully submit that the specification therefore clearly teaches how these conditions, by permitting identification of the recited activities, permit identification of whether or not a candidate agent alters these activities, thereby to identify an agent that alters mitochondrial function. Nevertheless, solely to advance prosecution without acquiescing in the rejection, and to make explicit what was implicit, independent claims 1, 19-21, 43, 64, 80 and 81 have been amended to recite expressly that conditions that permit identification of mitochondrial calcium uniporter activity are present when a decrease over time in the signal that is proportional to the level of calcium in the cytosol is detected, and that conditions that permit identification of mitochondrial uncoupler or respiratory inhibitor activity are present when an increase over time in the signal that is proportional to the level of calcium in the cytosol without repeating the step of contacting a biological sample with a source of calcium cations is detected.

In particular, and as clearly described in the specification, according to the subject invention method, mitochondrial calcium uniporter activity corresponds to a stable decrease in the detectable signal generated by the calcium indicator molecule, which acts as a reporter of cytosolic (not mitochondrial) calcium levels. Such a decrease results from mitochondrial uptake and sequestration of calcium via the mitochondrial calcium uniporter (*e.g.*, page 17, lines 1-4; Figure 4A; page 64, lines 26-30). Applicants therefore respectfully submit that the specification clearly teaches the meaning of conditions that permit identification of mitochondrial calcium uniporter activity.

The specification also clearly teaches the meaning of conditions that permit identification of mitochondrial uncoupler or respiratory inhibitor activity. As described, for example, at page 17, lines 4-10; in Figure 4B; and at page 65, lines 5-19, the presence of mitochondrial uncoupler or respiratory inhibitor activity corresponds to an increase in the detectable signal generated by the calcium indicator molecule even without a second (or successive, see, *e.g.*, page 64, lines 11-25; pages 76-82) step of contacting the sample with a source of calcium cations. Such an increase results from reversal of the mitochondrial calcium uniporter, such that calcium cations are conducted out of the mitochondria and back into the cytosol, and/or from other mechanisms by which mitochondrial uptake and retention of Ca^{2+} are

compromised. Accordingly, and contrary to the assertion found at page 4, paragraph B of the Action, it is quite clear whether and when mitochondrial uniporter and uncoupler or respiratory inhibitor activities are measured according to the claimed methods.

In view of the foregoing, applicants respectfully submit that the application satisfies all requirements of 35 U.S.C. §112, second paragraph, and request that these rejections be withdrawn.

REJECTIONS UNDER 35 U.S.C. §103

Claims 1, 3, 4, 6-14, 16-22, 93 and 96 stand rejected under 35 U.S.C. §103 over Matlib et al. (*J. Biol. Chem.* 273:10223, 1998) in view of Litsky et al. (*Biochem.* 26:7071, 1997) in further view of Murphy et al. (*Proc. Nat. Acad. Sci. USA* 93:9893) and Marban et al. (U.S. Pat. No. 6,183,948). More specifically, the PTO alleges that Matlib et al. teach the effects of Ruthenium Red and Ru360 on mitochondrial calcium uptake, but concedes that Matlib et al. fail to disclose a calcium indicator molecule, conditions to detect mitochondrial uniporter, uncoupler or respiratory inhibitor activity, or high throughput assays. The PTO then alleges that Litsky et al. teach an assay for mitochondrial calcium uniporter activity, conditions for uncoupling mitochondria, and isolation of mitochondria. Murphy et al. are asserted by the PTO to teach a calcium indicator molecule (calcium green-5N) and mitochondrial Bcl-2 expression, while Marban et al. are alleged to teach high throughput screening of chemicals for mitochondrial effects. The PTO asserts that it would have been obvious at the time of filing the instant application to combine the assay of Litsky et al. with the teachings of Matlib et al. to study uniporter activity in a whole cell, and further to apply calcium green-5N of Murphy et al. to detect real-time calcium concentrations using high throughput methods of Marban et al.

Claims 2, 5, 43-56, 58 and 59 stand rejected under 35 U.S.C. §103 over Matlib et al. (*J. Biol. Chem.* 273:10223, 1998) in view of Litsky et al. (*Biochem.* 26:7071, 1997), Murphy et al. (*Proc. Nat. Acad. Sci. USA* 93:9893) and Marban et al. (U.S. Pat. No. 6,183,948) in further view of McCormack et al. (*Biochim. Biophys. Acta* 973:420, 1989). PTO assertions regarding Matlib et al., Litsky et al., Murphy et al. and Marban et al. are summarized above. The PTO asserts further that McCormack et al. teach the use of ionomycin to equilibrate extramitochondrial and mitochondrial matrix calcium levels, alleging that it would have been

obvious to apply repeated contact with increasing calcium cation of McCormack et al. to the assay of Matlib et al.

Claims 80-91 stand rejected under 35 U.S.C. §103 over Litsky et al. (*Biochem.* 26:7071, 1997) in view of Murphy et al. (*Proc. Nat. Acad. Sci. USA* 93:9893) in further view of Marban et al. (U.S. Pat. No. 6,183,948). The PTO asserts that Litsky et al. teach mitochondrial calcium uniporter activity by adding EGTA and magnesium cation to isolated mitochondria, and that Litsky et al. teach mitochondrial uncoupling with CCP. PTO assertions regarding Murphy et al. and Marban et al. are discussed above. The Action alleges that it would have been obvious to use calcium green-5N of Murphy et al. in the assays of Litsky et al. and Matlib et al., and further that an ordinarily skilled artisan would have been motivated to apply a high throughput screen of Marban et al. to the assay of Litsky et al.

Claims 94-95 stand rejected under 35 U.S.C. §103 over Matlib et al. (*J. Biol. Chem.* 273:10223, 1998) in view of Litsky et al. (*Biochem.* 26:7071, 1997), Murphy et al. (*Proc. Nat. Acad. Sci. USA* 93:9893) and Marban et al. (U.S. Pat. No. 6,183,948) in further view of Bernardi et al. (*J. Biol. Chem.* 268:1005, 1993). PTO assertions regarding Matlib et al., Litsky et al., Murphy et al. and Marban et al. are discussed above. The Action alleges that Bernardi et al. "teach the effect of cyclosoporin on MTP transition pore with Ca++ ions", asserting specifically that one of ordinary skill in the art would have been motivated to use cyclosporin of Bernardi et al. in the assay of Matlib et al. to examine the "MTP inhibitory effect" on calcium uptake.

Applicants respectfully traverse these grounds for rejection and submit that the PTO has failed to establish a *prima facie* case of obviousness. The present invention is directed in pertinent part to a method of identifying an agent that alters mitochondrial function, comprising (a) contacting a biological sample with a source of calcium cations under conditions that permit maintenance of mitochondrial membrane potential, the sample comprising a cell containing cytosol, a mitochondrion and a calcium indicator molecule that generates a detectable signal that is proportional to cytosolic calcium levels; (b) detecting the signal at a plurality of time points under conditions that permit identification of (i) mitochondrial calcium uniporter activity, wherein a decrease over time in the signal that is proportional to the level of calcium in the cytosol indicates mitochondrial calcium uniporter activity, and (ii) mitochondrial uncoupler or respiratory inhibitor activity, wherein an increase over time in the signal that is proportional to

the level of calcium in the cytosol without repeating (a) indicates mitochondrial uncoupler or respiratory activity; and (c) comparing the signal generated by the calcium indicator molecule at one or more of said time points in the absence and presence of a candidate agent.

According to the present invention, the recited steps of contacting, detecting and comparing at one or more time points in the *absence* of a candidate agent provide an opportunity for mitochondrial uptake and sequestration of calcium cations, as described in the specification, for example, at page 17, lines 1-16; at page 61, lines 13-22; and at page 64, line 26 through page 66, line 2; and as detailed above. As also discussed above, when the recited steps of contacting, detecting and comparing at one or more time points are performed in the *presence* of the candidate agent, conditions are provided that permit identification of (i) mitochondrial calcium uniporter activity, and (ii) mitochondrial uncoupler or respiratory inhibitor activity. Specifically, a decrease over time in the signal that is proportional to the level of calcium in the cytosol indicates mitochondrial calcium uniporter activity, and an increase over time in the signal that is proportional to the level of calcium in the cytosol without repeating the step of contacting indicates mitochondrial uncoupler or respiratory activity. Applicants submit that neither Matlib et al. nor any combination of the cited publications in any way suggests this feature of the claimed invention. At best, the cited documents disclose several, but certainly not all, of the elements that are present in the claimed assay methods. Therefore applicants submit that the combination of these cited publications falls far short of in any way suggesting the particular combination which is the present invention, nor can there be any reason to believe that a person having ordinary skill in the art would have been motivated to successfully combine the cited articles to arrive at the present invention without the teachings of the present application.

Matlib et al. merely describe inhibition by Ru360 and Ruthenium Red, known calcium uniporter inhibitors, of mitochondrial calcium uptake under conditions that cannot distinguish between calcium uniporter activity and mitochondrial uncoupler or respiratory inhibitor activity. Matlib et al. fail, however, to teach or suggest a method comprising the recited steps of contacting, detecting and comparing. Specifically, Matlib et al. fail to teach or suggest the recited high throughput method comprising contacting a sample comprising a cell containing cytosol, a mitochondrion and an indicator of cytosolic calcium, with a source of calcium cations;

detecting a cytosolic calcium signal at a plurality of time points under conditions that permit identification of mitochondrial calcium uniporter activity and that permit identification of mitochondrial uncoupler or respiratory inhibitor activity; and comparing the signal that is generated in the absence of a candidate agent to the signal generated in the presence of the candidate agent. Applicants submit that, for reasons elaborated upon below, the other cited references fail to cure these deficiencies of Matlib et al.

According to the method of Matlib et al., it would not be possible to distinguish between mitochondrial calcium uniporter activity and mitochondrial uncoupler or respiratory inhibitor activity, because Matlib et al. fail in any way to contemplate monitoring calcium cation distribution between mitochondrial and cytosolic compartments at a plurality of time points, at least one or more of which occurs in the absence of a candidate agent (*e.g.*, Ru360). Thus, the method of Matlib et al. fails to provide for the mitochondrial calcium sequestration or "loading" event that permits identification of the presently recited mitochondrial activities. Matlib et al., in combination with the other cited publications, fail to suggest conditions for such identification, nor is the complete combination of all elements of the instant claims even remotely suggested by the cited documents. As discussed above, the subject invention method relates to time-course monitoring of cytosolic calcium levels, which can decline as mitochondrial calcium uniporter activity sequesters calcium intramitochondrially (*e.g.*, application Figure 4A), or which can rise without repeating the step of contacting if at a later time point a candidate agent is present that causes uncoupler or respiratory inhibitor activity (*e.g.*, application Figure 4B). The invention thus provides unexpected advantages in high throughput screening for *bona fide* mitochondrial calcium uniporter inhibitors or stimulators (*e.g.*, application Figure 4D) in a method that permits identification (*e.g.*, and elimination) of uncouplers and inhibitors (Fig. 4B).

With regard to the specific assertion in the Action at page 5, lines 9-10, that Matlib et al. at page 10224 describe digitonin permeabilized cells (apparently a reference to instant claim 16), applicants submit that in this regard Matlib et al. also fail to teach or suggest, *inter alia*, a method that employs a biological sample comprising a cell containing a mitochondrion under conditions that permit maintenance of mitochondrial membrane potential. In particular, the digitonin permeabilized cells of Matlib et al. were assayed in the presence of the respiratory inhibitors rotenone and oligomycin (Matlib et al., page 10224, right-hand column,

lines 56-57), which are well known in the mitochondrial art to impair mitochondrial membrane potential. By contrast, the instant claims recite "conditions that permit maintenance of mitochondrial membrane potential" and the specification clearly describes how to determine such conditions, for example, at page 36, lines 14-27. Neither Matlib et al. nor the other cited references teach or suggest combining such permeabilized cells under conditions that permit maintenance of mitochondrial membrane potential with conditions that permit identification of mitochondrial calcium uniporter and uncoupler or respiratory inhibitor activity in a high throughput screening assay according to the instant claims, nor has the PTO specifically pointed to any suggestion to make such combination.

As noted above, the PTO concedes that Matlib et al. fail to disclose a calcium indicator molecule, conditions to detect mitochondrial uniporter, uncoupler or respiratory inhibitor activity, or high throughput assays, and for reasons given herein any combination of the publications cited in the Action fails to remedy the deficiencies of Matlib et al.

Applicants respectfully submit that the disclosure of Litsky et al. is inapposite to the present invention and would not have motivated a person having ordinary skill in the art to combine any teachings found therein with any other prior art documents, to arrive at the presently claimed invention with any reasonable expectation of success. The disclosure of Litsky et al. relates exclusively to studies performed using isolated mitochondria or mitoplasts derived from such mitochondria, while the instant claims relate to the use of a biological sample comprising a cell containing cytosol, a mitochondrion and a calcium indicator molecule. Litsky et al. fail to teach or suggest detecting a signal that is proportional to the level of calcium in cytosol; cytosol is not even present in any samples of Litsky et al. The present invention, as noted above, relates to a biological sample comprising a cell containing cytosol, and to detection of a signal that is proportional to the level of calcium in the cytosol. Litsky et al. employ no calcium indicator molecule and do not even detect calcium. Rather, Litsky et al. merely monitor strontium cation and not calcium cation.

Moreover, throughout the disclosure of Litsky et al. the mitochondrial uncoupler CCP and the mitochondrial electron transport chain inhibitor rotenone are present. As noted above, such compounds as presented by Litsky et al. preclude maintenance of mitochondrial membrane potential. By contrast, the method of the instant claims includes the recited step of

contacting a biological sample with a source of calcium cations under conditions that permit maintenance of mitochondrial membrane potential. Accordingly, where Litsky et al. fail to contact a sample with calcium cations, where Litsky et al. fail to employ a calcium indicator molecule and fail to detect a calcium signal generated by such molecule, where Litsky et al. fail to suggest conditions by which calcium uniporter activity and mitochondrial uncoupler or respiratory inhibitor activity can be identified, and where Litsky et al. fail to suggest a high throughput screening assay for identifying an agent that alters mitochondrial function, applicants submit that the PTO has failed to provide evidence or reasoning to suggest that a person having ordinary skill in the art would in any way have been motivated to combine the teachings of Litsky et al. with any other document to arrive at the instant invention.

Applicants submit further that Murphy et al., alone or in combination with Matlib et al., Litsky et al. and/or Marban et al., fail to anticipate the subject matter of the instant claims. Murphy et al. disclose that Bcl-2 expressing cells, including cells expressing a Bcl-2-encoding transgene, exhibit enhanced ability to sequester calcium in mitochondria. Murphy et al. also describe the use of calcium green-5N to determine calcium levels; use of calcium green-5N to determine calcium was previously known (see reference 37 in Murphy et al.). However, Murphy et al. fail to contemplate, *inter alia*, use of the method described therein for screening candidate agents in a method of identifying an agent that alters mitochondrial function, nor do Murphy et al. teach or suggest a high throughput screening array. Applicants therefore further respectfully submit that the disclosure of Murphy et al. includes no teaching or suggestion that any assay described therein is desirably designed to include conditions that permit identification of (i) mitochondrial calcium uniporter activity and (ii) mitochondrial uncoupler or respiratory inhibitor activity, and in particular, that Murphy et al. fail to provide for determining whether an agent that could be screened according to the teachings therein might influence either of these activities. As these deficiencies of Murphy et al. are not remedied by Matlib et al., Litsky et al. and Marban et al. for reasons set forth herein, and in particular where the Action fails to point to any suggestion in any of the cited references to combine its teachings with those found in any other of the cited references or known to the art to achieve the instant invention, applicants submit that the Action has failed to establish a case of *prima facie* obviousness.

The disclosure of Marban et al. relates generally to assays of mitochondrial function, including screening assays, but applicants submit that given the teachings of Matlib et al., Murphy et al., Litsky et al. and Marban et al., a person having ordinary skill in the art at the time of filing the instant application would not have been motivated specifically to arrive at the presently claimed invention with any reasonable expectation of success.

Marban et al. merely describe screening assays that comprise monitoring changes in endogenous cellular fluorescence that result from alterations in a mitochondrial redox state within cells. Marban et al. fail, however, to teach or suggest a method comprising the use of a calcium indicator molecule that is capable of generating a detectable signal that is proportional to the level of calcium in a cell, in cytosol or in a mitochondrial medium as provided by the present invention, nor does the Action anywhere point to such a suggestion in Marban et al. The assays of Marban et al. are not specific assays of intracellular calcium levels, and certainly cannot be specific assays of mitochondrial calcium transport activity such as a mitochondrial calcium uniporter activity and mitochondrial uncoupler or respiratory inhibitor activity, because the endogenous fluorescent redox indicator disclosed by Marban et al. may generate a signal indicative of altered redox conditions in response to any of a number of non-specific stimuli that are wholly independent of the mitochondrial calcium uniporter. Applicants respectfully submit that neither do Marban et al. suggest the desirability of combining the teachings therein with Matlib et al. or with any other reference known to the art, to arrive at the presently claimed invention, especially where these references or any other disclosures known to the art fail to provide assay conditions that permit distinguishing mitochondrial uncouplers or respiratory inhibitors from mitochondrial calcium uniporter activity inhibitors. The combination of Marban et al., Litsky et al., Murphy et al. and Matlib et al. is silent with regard to assay conditions (including, *e.g.*, the choice of calcium indicator molecule) that permit identification of mitochondrial calcium uniporter activity and of mitochondrial uncoupler or respiratory inhibitor activity in a high throughput screen. In other words, and as discussed above, absent the teachings of the present application a person having ordinary skill in the art would not, with any reasonable expectation of success, have been able to select a calcium indicator molecule and conditions for distinguishing between (i) a candidate agent that alters mitochondrial or extramitochondrial calcium levels by altering mitochondrial calcium uniporter activity, and (ii) a

candidate agent that alters mitochondrial or extramitochondrial calcium levels by having mitochondrial uncoupler or respiratory inhibitor activity. Applicants submit that the Action employs impermissible hindsight in view of the instant application to assert otherwise, especially where the Action fails specifically to point to any suggestion in any of the cited references to combine elements known to the art, to arrive at the claimed invention.

Applicants submit further that McCormack et al. fail to remedy the deficiencies of Matlib et al. and Marban et al. because, *inter alia*, McCormack et al. only describe assays that are *not* conducted under conditions that permit maintenance of mitochondrial membrane potential; the teachings of McCormack et al. are also limited to assays using isolated mitochondria. Moreover, McCormack et al. fail to teach or suggest a screening method wherein detection of a signal generated by a calcium indicator molecule is performed under conditions that permit identification of mitochondrial calcium uniporter activity and mitochondrial uncoupler or respiratory inhibitor activity. Thus, like Matlib et al. and Marban et al., McCormack et al. fail to anticipate the instant invention where no assay described therein would have motivated an ordinarily skilled artisan to arrive at the subject invention assay method, which permits such mechanistic characterization (*i.e.*, uniporter inhibitor *vs.* uncoupler/ respiratory inhibitor) of a candidate agent based on the detected signal.

McCormack et al. describe fluorescence experiments using isolated rat heart mitochondria under conditions in which the mitochondria are not permitted to maintain mitochondrial membrane potential. McCormack et al. employ a calcium-sensitive fluorescent indicator molecule and expose the mitochondria to ionomycin to equilibrate the mitochondrial matrix and extramitochondrial Ca^{2+} pools. As described by McCormack et al., ionomycin treatment selectively permeabilizes mitochondria to calcium cations, thereby overriding any endogenously regulated mitochondrial calcium transport activity such as mitochondrial calcium uniporter activity. As well, McCormack et al. describe the use of a mitochondrial uncoupler and thereby cause dissipation of mitochondrial membrane potential. By way of contrast, according to the present invention the recited step of contacting the sample with a source of calcium cations takes place under conditions that permit calcium uniporter activity to take place, thereby permitting agents that alter calcium uniporter activity to be identified. Thus, while certain embodiments of the present invention relate to the use of permeabilized *cells*, the claimed

methods do not contemplate *mitochondria* that are artificially rendered calcium-permeable according to the disclosure of McCormack et al.

Applicants therefore submit that if anything, the disclosure of McCormack et al. teaches away from the presently claimed invention because the cited publication teaches that the presence of ionomycin precludes detection of mitochondrial calcium uniporter activity, while the instant claims are directed to a method comprising the recited step of detecting under conditions that permit identification of mitochondrial calcium uniporter activity. Therefore, applicants submit that it would not have been *prima facie* obvious to combine the teachings of McCormack et al., Marban et al., Murphy et al., Litsky et al. and Matlib et al in order to screen a plurality of samples for their effect on mitochondrial function.

Bernardi et al., alone or in combination with any or all of the other cited publications, fail to render obvious claims 94-95 or any other claims of the instant application. Bernardi et al. merely disclose the effects of calcium cations and uncouplers on permeability transition in isolated rat liver mitochondria, where Bernardi et al. only describe assays in which mitochondrial membrane potential or mitochondrial matrix pH are measured. Specifically, Bernardi et al. demonstrate that cyclosporin A can inhibit mitochondrial permeability transition (MPT) under certain conditions. The disclosure of Bernardi et al. is therefore merely cumulative with disclosure in the present application of compounds, including cyclosporin, that alter mitochondrial function and which may optionally be included in certain embodiments of the invention (*e.g.*, specification at page 21, line 13). Even combined with Matlib et al., Litsky et al., Marban et al. and/or Murphy et al., however, Bernardi et al. fail to contemplate the subject invention assay method, in which a single set of assay conditions permits an ordinarily skilled artisan to distinguish between a candidate agent that alters mitochondrial calcium uniporter activity and a candidate agent that has mitochondrial uncoupler or respiratory inhibitor activity, by detecting the signal generated by a calcium indicator molecule.

Accordingly, applicants submit that even in view of the other references cited in the Action, the mere disclosure by Bernardi et al. that cyclosporin A influences MPT, where an association between calcium cations and MPT was long known (*e.g..*, specification at page 3, line 14 through page 4, line 19; page 23, lines 10-30), fails to anticipate the instant claims. Applicants therefore traverse the assertion in the Action and submit that no *prima facie* case of

obviousness has been established, where the Examiner has provided neither evidence nor reasoning to support the allegation that the cited references would have motivated the ordinarily skilled artisan to arrive at the instant invention absent the disclosure of the present application. In particular, and for reasons discussed in greater detail above, simply combining cyclosporin A in the assay of Matlib et al. would fall far short of any suggestion of the present invention, where Matlib et al. fail to contemplate a method comprising, *inter alia*, the recited step of detecting a signal generated by a calcium indicator molecule under conditions (including the choice of calcium indicator molecule) that have been selected to permit identification of mitochondrial calcium uniporter activity and of mitochondrial uncoupler or respiratory inhibitor activity.

Applicants therefore respectfully submit that the documents cited by the PTO, alone or in combination, fail to teach or suggest the subject matter of the instant claims, and that the PTO has not established a *prima facie* case of obviousness. (*See In re Mayne*, 104 F.3d 133, 1341-43, 41 U.S.P.Q.2d 1451 (Fed. Cir. 1997) (PTO has the burden of showing a *prima facie* case of obviousness.)). The PTO must show (1) that the combined references teach or suggest all claim limitations; (2) that the references provide some teaching, suggestion, or motivation to combine or modify the teachings of the prior art to produce the claimed invention; and (3) that the combined teachings of the references indicate that by combining the references, a person having ordinary skill in the art will achieve the claimed invention with a reasonable expectation of success. When rejection of claims depends upon a combination of prior art references, a teaching, motivation, or suggestion to combine the references must exist. (*See In re Rouffet*, 149 F.3d 1350, 1355, 47 U.S.P.Q.2d 1453 (Fed. Cir. 1998)). For reasons given herein, no such teaching, motivation or suggestion to combine the references can be found in the prior art.

Applicants also respectfully submit that the mere fact that the teachings of the prior art *can* be combined or modified, or that a person having ordinary skill in the art is *capable* of combining or modifying the teachings of the prior art, does not make the resultant combination *prima facie* obvious, as the prior art must also suggest the desirability of the combination (*see, e.g.*, *In re Mills*, 16 U.S.P.Q.2d 1430, Fed. Cir. 1990; *In re Fritch*, 23 U.S.P.Q.2d 1780, Fed. Cir. 1992). Thus, applicants submit the cited references, taken alone or in combination, fail to provide any motivation or suggestion to a person of ordinary skill in the art

to combine or modify the references to arrive at the claimed invention. Accordingly, applicants respectfully submit that the instant claims satisfy the requirements of 35 U.S.C. § 103(a) and request that these rejections be withdrawn.

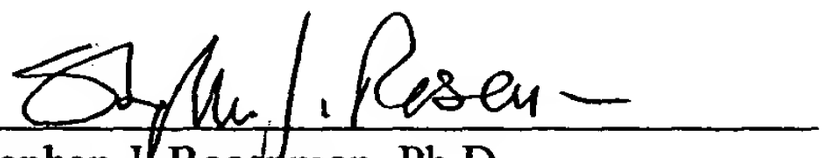
The Commissioner is authorized to charge any additional fees due by way of this Amendment, or credit any overpayment, to our Deposit Account No. 19-1090.

All of the claims remaining in the application are now clearly allowable. Favorable consideration and a Notice of Allowance are earnestly solicited.

Respectfully submitted,

Anne N. Murphy and Amy K. Stout

SEED Intellectual Property Law Group PLLC



Stephen J. Rosenman, Ph.D.
Registration No. 43,058

SJR:kw
Enclosures:
Postcard
Check
11 Sheets of Formal Drawings (Figs. 1-13B)
Petition for Extension of Time

701 Fifth Avenue, Suite 6300
Seattle, Washington 98104-7092
Phone: (206) 622-4900
Fax: (206) 682-6031

D:\NrPortbl\iManage\KARENWILL\373271_1.DOC